Germination and Expression of Hydrolysing enzymes in soybean and maize seeds after pretreatment with magnetic field and Plasma

A Synopsis Submitted to

Shri Vaishnav Vidyapeeth Vishwavidyalaya

IN

LIFE SCIENCE

By

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Under the Guidance of

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FACULTY OF DOCTORAL STUDIES AND RESEARCH

Report of the Research Progress Committee

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Proposed Topic: Germination and Expression of Hydrolysing Enzymes in Soybean and Maize Seeds After Pretreatment With Magnetic Field and Plasma

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Observations

About the Topic: Good

Review of Literature: Reasonable

Research Method: should explain more

References: Good

General: Good

✓

Approved /Not Approved with Specific Reasons

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PhD Thesis Synopsis

Title

"Germination and Expression of Hydrolysing enzymes in soybean and maize seeds after pre treatment with magnetic field and Plasma."

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1. Introduction:

A seed is an embryonic plant encased in protective external covering. It has the capacity to develop on-ranch productivity and for refreshing nourishment security [1]. Seed quality is essential establishment for productive generation and showcasing [2, 3]. Best quality seeds are hereditarily just as physically, unadulterated and free from pathogens [4]. The seeds with upgraded quality contribute almost 30% of the total production. A seed enhancement includes physical, physiological and natural treatments to enhance germination which results in the better yields.

Germination is seen as a response which joins bidirectional relationship between the Embryo and endosperm since the endosperm can discharge signs to control embryo development. It is the process of initiating growth of a dormant embryo. Enhanced germination rate and seedling vitality are because of diminished development time by promising start of metabolic activities of hydrolytic catalysts and resources activation.

Seed germination is basic stage in plant improvement and can be considered as a determinant for plant effectiveness. It begins by water imbibitions, assembly of food reserve, protein amalgamation and outcome radical distension [5].During germination physiological and biochemical changes are followed by morphological changes and are firmly identified with seedling survival rate and vegetative development which impact yield and quality.

Water imbibitions by reserved substances in growing seed fortifies the developing life to create phytohormones for the most part gibberellic acid(GA) which can diffuse to aleurone layer besides, begin a flagging course realizing the association

of α-amylases and other hydrolytic Compounds. At that point, hydrolytic enzymes secrete into the endosperm and hydrolyze food reserves [6, 7].

1.1. The job of hydrolytic enzymes in seed germination:

Separate intercellular assortments of seed which stores starches, proteins, lipid and, phosphate which acts about as imperativeness source and carbon skeleton on seed hydration [8]. Seed imbibition actuate various metabolic techniques, for instance, initiation or union of new hydrolytic chemicals which achieve hydrolysis of stored starch, lipid, protein hemicellulose, polyphosphates and other stockpiling materials into fundamental accessible structure for incipient embryo take-up. Likewise, utilization of a raised dimension of oxygen may instigate actuation institution/hydration of mitochondrial chemicals, occupied with the Krebs cycle and electron transport chain [9, 10].

1.2. Seed Enhancement:

Enhancement of Seed and seed upgrading are the post- harvest treatments utilised for the betterment of germination as well as advancement of seedlings required at the period of sowing [11]. Different methodologies are being utilized for improvement of seeds all through the past twenty years, which includes seed preparing, magnetic stimulation, seed capping, and treatment by plasma etc. [11, 12, and 13].

Physical treatments are connected externally with no hydration and without use of any chemicals to the seeds. The main reason is to enhance germination as well as seedling foundation Among physical treatment, magnetic field and illumination with microwave or ionizing radiations are the best promising pre-sowing

medications for seed [14].Therefore, physical seed treatments are the optional solution to manage the chemical seed invigoration treatments, which gives good response for the developing scene of seed technology.

1.3. Magnetic fields for seed treatments:

Magnetic seed stimulation incorporates recognizing magnetic dose for influencing the germination rate, early seedling improvement as well as subsequent yield of crops [15]. The magnetic introduction dose is result of motion thickness of magnetic field and for the certain interval of introduction of time. The transition thickness of magnetic field varies with the static or pivoting magnetic fields exposure to seed. Magnetic field guarantees uniform crop stand establishment, fast germination, furthermore, yield of different agronomic and plant crops [16, 17]. These won't simply expand the rates of germination, yield and improvement [18] however what's more reducing the pathogenic attack [8, 9].

Introduction to magnetic field expands germination of non-standard seeds and furthermore improves their quality. Magnetic field impacts the underlying creating period of the plants after germination [10]. In present occasions, work at magnetically treated water uncovers that the plant headway and seed germination gets improved by preparing them [19].

1.4 Plasma seed treatment:

Plasma applications in horticulture and therapeutic field is an ongoing headway [20– 23]. The farming points of view incorporate seed germination just as plant improvement. A few reports say that germination and development advancement component is affected by the use of plasma with a couple of gases, for example, aniline, cyclohexane and helium [24, 25]. For refreshing seed advancement and plant development atmospheric pressurized plasma, microwave plasma, polarised

plasma are ongoing treatments [26, 27]. The impact of gases is much typically observed in plasma treatments. Some reports revealed that, nature of seeds which controls thiol groups are upgraded by redox response which in addition influenced by the dynamic oxygen types of water vapor plasma [28]. Non-warm plasmas are utilized in agricultural practices as option in contrast with stratification, scarification and priming which helps in improving the plant development [29]. Plasmas establishes zero seed decimation, no chemical use and conditions welcoming treatments for seeds [24, 29, and 30].Plasma treatment improves quality of seed and plant development [26, 31]. Exposing seeds to plasma moreover brings about some changes in action of enzymes [28] and causes disinfecting of seed surfaces [30]. Plasmas can tune seed germination by deferring and boosting with the usage of plasma-treated testimony on seed surfaces [24]. The constant noteworthy plasma-related examination teaches the demonstration of microwave release [26] just as low-thickness radio recurrence (RF) discharges [32, 33]. The release of atmospheric pressure and the arrival of coplanar barrier have been surveyed in progressing studies [24, 31]. The examination of various seed germination designs is actualized on various seeds including wheat, maize, blue lupin radish, and oat, safflower [26, 29, 31, and 33]. Safflower seeds expression contributed over more than half of the rate of germination when treated with the radio frequency plasmas for 130 min with argon gas [29].likewise, Soybean seeds are treated with the cold plasma treatment with 0, 60, 80, 100 and 120 W for 15 s and better consequences of cold plasma treatment on germination of seed and seedling growth of soybean has been obtained [34].

2. Objective:

Perusal of literature reveals that least work has been done in India and abroad regarding the expression of hydrolysing enzymes in seeds and the after effects of physical treatments (magnetic field and plasma treatment) on seeds. Hence in the present proposal “A study on germination and expression of hydrolyzing enzymes after treatment with magnetic field and plasma” a detailed study will be undertaken on the activity of the hydrolyzing enzymes and their expression. The role of ROS

[reactive oxygen species] will also be assessed in the promotion of germination by these two physical treatments.

3. Rationale of the study:

There have been some preliminary studies done in our laboratory on the biochemical changes induced by magnetic field treatment of seeds. Treatment with low magnetic fields induces proportion of reactive oxygen species (35) and nitrous oxide (36). Besides this magnetic field treatment also enhances amylase activity in maize. However the impact of these biochemical changes on the expression of genes for hydrolyzing enzymes is yet to be expressed. Since germination is enhanced by both magnetic field and plasma treatment of seeds, there is possibility that biochemical events preceding germination may be similar in both the treatments. The present study will also bring out the signal transduction events in plasma treatment which is known to generate ROS.

4. Methodology:

Soybean (Glycine max.) member of family Fabaceae and Maize (zea mays) member of family Poaceae, have been selected for this study since the impact of magnetic field on these two types of seeds has been worked out in our laboratories.

The good quality seeds of maize and soybean with uniform shape and size without visible defects and malformation will be used in the study. A Few local varieties of soybean and maize will be initially screened for their response to magnetic field and plasma treatment and the best responding variety will be selected for detailed biochemical study.

4.1. Plasma and magnetic field devices and generation.

A Testron EM-20 electromagnetic field generator (Testron Instruments, Delhi, India) will be used with a variable even magnetic field strength (50– 500 mT) and a hole of 5 cm between pole pieces. A computerized gauss meter (Model DGM-30, Testron Instrument, Delhi, India), working on the rule of the Hall Effect will be used for measuring field strength.

In this investigation, for plasma treatment, twofold dielectric obstruction release, In two diverse setup, will be utilized for water and seed treatment: (I) plate-to-plate twofold DBD (PtP-DBD) reactor and, (ii) barrel formed twofold DBD (Cyl-DBD) reactor.The Fig. 1 depicts the schematic diagram of the reactor for treatment of the seed.

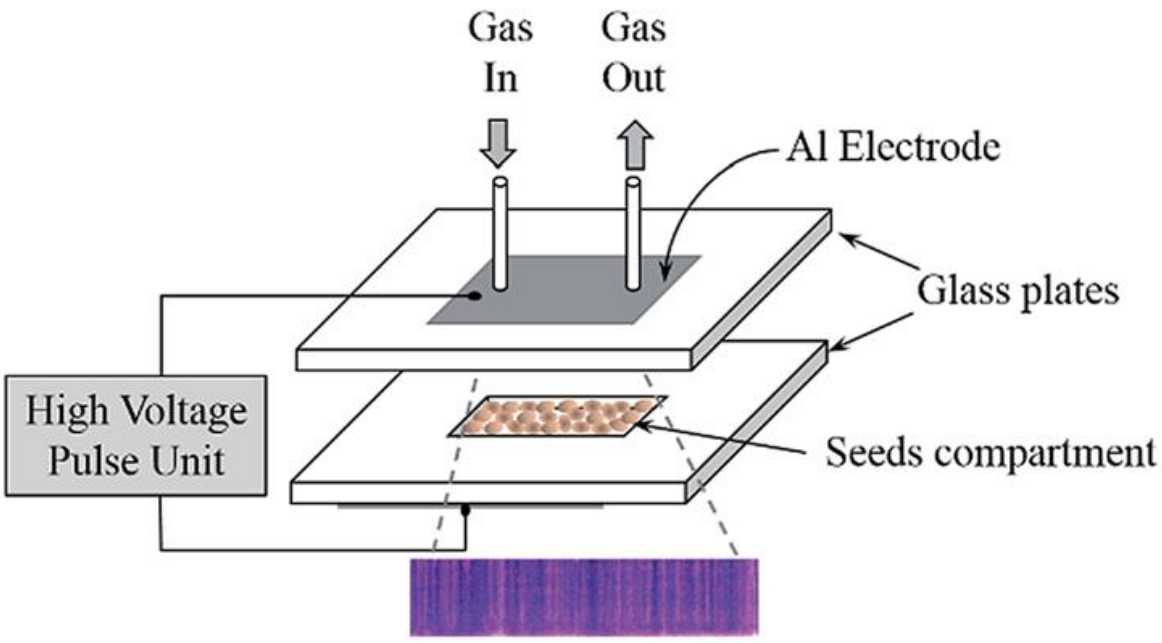

Fig. 1: Plasma reactor setup for seed treatment

4.2. Germination:

Magnetic field treatment and plasma treatment will be given at pre decided intervals of time. Seed germination will be determined by following the method of international seed testing (ISTA 1985). Percent germination of seeds and seedling vigour will be measured initially in all the varieties of soybean and maize for the purpose of comparison and selection of the best responding variety.

4.3. Biochemical analysis:

Analysis of superoxide production by EPR (Electron Para magnetic resonance spectrometer) will be done by the method of *shine et. Al (*2012*) (35)*. Hydrogen peroxide content will be measured spectrophotometrically by the method of Mukherjee and chowdhury 1983(37). Ascorbate will be determined by the method of Arakawa et.al (1981)(38).

Superoxide dismutase (SOD) guaiacol peroxide (Gpox) will be extracted by the method of Giannopolisis and Rics (1977) (39). Gpox activity will be assayed by the method of chance and Maehly (1955) (40). Ascorbic acid peroxidase will be assayed by the method of Nakano and Asada (1981) (41). The total protein content will be measured by the method of Lowry et.al (1951) (42). Glutathione reductase activity will be determined by the method of Rao et.al (1996) (43). Amylase activity will be measured as per the method of shawney et.al (1970) (44).

4.4. Molecular Biology:

Isolation of RNA, c- dna synthesis and real time PCR will be done by the method as described by schmittigen (2008)(45).

4.5. Statistical analysis:

Complete data collected throughout the study will be analyzed statistically for better presentation and understanding of the results .Minor changes could be made in the plan if necessary or required.

5. Possible Outcomes:

Plasma and magnetic field treatments give impact on different crop. This study will be useful for checking the effects as well as role and modifications with the activity of hydrolyzing enzymes. Pre-sowing treatment of seeds with magnetic field has valuable impacts. Exposure of seeds to magnetic field is one of the potential, and moderate physical pre-sowing treatment to upgrade post germination plant advancement and crop stand. This can possibly build crop Generation per unit zone of land without having any harming impact towards environment. Additionally, MF pretreated seeds might be viably Utilized for relieving of abiotic stresses like water pressure, saltiness, high temperature and, UV-B radiations.

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